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INTERACTIONS BETWEEN MOSQUITO REPELLENTS AND HUMAN SKIN.(U)

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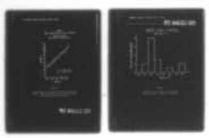

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Dry protection time afforded by mosquito repellents is shown to depend on individual human differences and repellent evaporation rate. Data for repellent protection for 30 young adult males have been accumulated for a period of two years. Protection times afforded by deet are normally distributed with individuals maintaining their relative ranks within the population consistently.

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INTERACTIONS BETWEEN MOSQUITO REPELLENTS AND HUMAN SKIN

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Malaria, yellow fever, dengue, and other mosquito-borne diseases become a major problem when military forces must enter areas where mosquitoes have not been controlled. Consequently, some means of personal protection is necessary when the exposed individuals do not have natural or acquired immunity to these diseases. To date, most efforts to develop an effective mosquito repellent have been directed toward the chemical aspects of repellency (1,2) rather than the physical and biological aspects.

To investigate the behavior of repellents on man's skin and the importance of the physical properties of the repellent in protection from mosquitoes, a repellent testing program is being carried out at the Department of Dermatology Research, Letterman Army Institute of Research (LAIR). By employing a closed group of research subjects, we have studied the biological variations present in repellent protection and the relative effect of the evaporation rate of repellents.

Some factors affecting mosquito repellent protection times include the species, the avidity, and the density of the mosquito population.

Climatic conditions like temperature and relative humidity are known to affect both the mosquito population and man.(3,4) Warm moist climates tend to increase mosquito avidity and density (3) and

to make the individual both more attractive due to sweat (5,6) and more exposed because of thinner and scantier clothing. These conditions make the individual more susceptible to mosquitoes' biting. Sweating and abrasion are factors which are difficult to quantitate in repellent failure.

Repellent evaporation is subject to individual physical repellent-skin interactions. Possible synergistic effects between the repellent and the individual may occur which would prolong the protection time of the repellent compound. (6) Differences in repellent protection times have been attributed to differing rates of loss rather than differences in attractancy between individuals. (6)

The minimum effective dose (MED) which will repel mosquitoes has been reported to vary only slightly among individuals. (6) The MED has been used historically as a measure of intrinsic repellency of a compound. Although the MED measures in part the intrinsic repellency of a mosquito repellent, (7,8) the evaporation rate of the repellent from the skin must also be considered in evaluating the repellency per molecule. The intrinsic repellency of a compound has been related to molecular structure; (1,2) however, the interaction of the repellent with the skin needs consideration.

This paper reports the human biological variation in mosquito repellent dry protection times and minimum effective repellent concentrations for several new repellents. Relative repellent protection is shown to be related to the in vitro evaporation rate of the repellent under ambient temperatures.

Materials and Methods

The repellents used in these tests are: (1) N,N-diethyl-m-toluamide (DEET), Eastman Chemicals, Practical Grade; (2) 2-ethyl-1,3-hexanediol (6-12), Eastman Chemicals, Practical Grade; (3) n-butane-hexamethylene-sulfonamide (sulfonamide) (9); (4) cyclohexamethylene-carbamide (carbamide) (10,11); and (5) 3,6,9-trioxapentadecan-1-ol (SRI-6) (12).

The volunteers used for all of these tests were healthy male, active duty military personnel with an average age of twenty-two years. All personnel were stationed at the Letterman Army Institute of Research. Informed consent was obtained before any part of the described procedures was carried out.

All dry protection times (DPT) were determined in the following manner (13): two 7 by 10 cm patches were marked off on each

volunteer's ventral forearm (total of four sites). Repellents were spread evenly within these test sites with a clean glass rod. The repellents were mixed with ethanol (95%) to provide the specified unit/area concentration desired. In this case, 0.32 mg/cm^2 was applied. The standard test (13) involved a three minute exposure to the test species; 250 avid female _Aedes aegypti_ mosquitoes held in a 1' x 1' x 1 1/2' test cage with three sides and top covered by #24 net. A site was considered to have failed when it received two or more bites during one three-minute period or one bite during each of two consecutive three-minute test periods occurring 1 hour apart.

In the MED procedure, four 70 cm^2 sites were again marked off on the subject's ventral forearm. Then a low dose rate of repellent was applied to one site in the configuration. If this site failed by the standard test, a higher concentration was applied to another site to bracket the MED. If the first application did not fail, a lower concentration was applied and tested. Using the first two applications to approximate the MED, the last two applications of different doses defined the exact MED for the individual.

The profile test was performed using the same standard dry protection test, except only one site was used in the four-site configuration. This site had a 0.32 mg/cm^2 concentration of DEET applied to it. Thirty subjects were used for each profile test. These subjects were all tested the same day and against the same population of mosquitoes. The standard two bite criteria for failure was used to determine dry protection times for these subjects (13).

Evaporation rates of repellents were determined gravimetrically by static evaporation from a 1.13 cm^2 aluminum planchet filled with repellent using a Cahn RTL millibalance coupled to a Honeywell 1 mV recorder. The evaporation rates were determined at five temperatures from 25 to 60° C in a temperature controlled incubator. All runs were corrected for electronic drift which was determined by tare runs at each temperature studied. To determine the energy of activation of vaporization of the repellent, the evaporation data were plotted using the Arrhenius relation of $\ln k$ vs $1/T$. (14)

Results and Discussion

The repellent dry protection times (DPT) of DEET for the volunteer population can be described by the histograms in Figures 1 and 2. Probit analysis of these tests indicates that the sample population has a normal distribution in terms of mosquito repellent DPT (Figure 3). The mean DPT's (Table 1) were 6.8 ± 1.9 hours for

DEET applied at 0.31 mg/cm^2 in February, and 7.1 ± 1.8 hours in October. These results are close to the yearly average DPT for DEET in all tests of 6.78 ± 0.81 hours. Hence, the population distribution for a mosquito repellent is a reproducible biological distribution.

The variation in DPT between individuals occurs although individuals were tested under conditions where abrasion was excluded as a major experimental variable. In addition, the differences are consistent over a period of time so that individuals can be identified as long, medium, or short duration in terms of DPT. The variation between individuals is also found in the MED for a repellent (Figure 4). The MED profile shown for DEET is similar in distribution to those for carbamide and SRI-6, although the average MED's are different as indicated in Table 2. MED depends on the intrinsic repellency, the evaporation rate, and the repellent-skin interaction for a given repellent as well as individual attractancy (7). Since by definition a repellent has some intrinsic repellency, the evaporation rate becomes the important factor in the length of protection afforded by a given compound.

The evaporation rates of the test repellents are shown in Table 3 with the DPT's for each repellent. The length of protection time for these repellents increases as the evaporation rate decreases, hence, the evaporation rate for a known repellent is an important physical property. The boiling points of all the repellents studied are similar (Table 3), thereby indicating that the volatility under ambient conditions has little relation to the boiling point as previously proposed (15). For a known repellent, the relative evaporation rate gives an indication of relative DPT. Moreover, these rates are readily determined _in vitro_.

The molecular structure of the repellents are indicated in Table 4. All show good to excellent protection against mosquitoes under dry conditions (1,2). Each repellent has both polar hydroxyl or carbonyl groups and nonpolar hydrocarbon character. In the case of DEET, when the N-ethyl groups are reduced to N-methyl groups, the repellent protection time is reduced. (15) The effect might be a factor of volatility, but on the other hand, a balance between polar and nonpolar grouping should enhance the interaction between the repellent and the skin, thereby reducing the rate of evaporation. In addition, the balance between polar and nonpolar properties might be necessary for interaction of the repellent molecule with hte mosquito sensory membranes.

The molecular structure and the evaporation rate of repellents can be evaluated in the laboratory, but the major factor in the

length of protection time is individual variability as indicated by the distribution profiles previously described. For a given repellent like carbamide, when compared to the standard military repellent DEET, a higher initial dose might be necessary to overcome the effects of a higher MED (7). At first glance, one might reject a repellent as inferior on the basis of a higher MED. Nevertheless, the protection time offered by a repellent with a low volatility can be much greater than DEET when an adequate dose is applied under field conditions regardless of the MED. Actual usage application rates are 5 - 10 times greater than the doses used in screening tests. The higher MED (Table 2) for carbamide is simply a reflection of a lower volatility of carbamide versus DEET; therefore, a higher initial concentration is necessary to put the requisite number of repellent molecules in the air to keep mosquitoes from landing on the skin.

In summary, a large variation in duration has been demonstrated by a given repellent. This variability also reflects the minimum effective amount of repellent necessary to repel mosquitoes. Furthermore, differences in DPT's between repellents have been related to the evaporation rates of the repellent which influences both the DPT and the MED. Finally, one would expect to obtain the longest protection from mosquitoes with a repellent which has an evaporation rate close to the threshold of repellent vapor or minimum inhibitory concentration which is necessary to repel mosquitoes.

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Table 1

DEET Profile Dry Protection Time[1]

	February	October	One Year Mean[2]
$\bar{X}$	6.8	7.1	6.68
S_d	1.9	1.8	0.81
Number of Subjects	32	32	
Skewness	0.17	0.43	0.15

[1]Dry protection time in hours for DEET applied at 0.31 mg/cm^2 using 4-patch method.

[2]Average of 12 tests with a total of 128 subject exposures from June 1972 to October 1973.

Table 2
Dry Protection Times for Test Repellents

	DPT (hrs)	MED (mg/cm^2)	Subject Exposures
DEET	6.68 ± 0.81	0.025 ± 0.02	128
6-12	3.40 ± 0.5*		40
SRI-6	8.50 ± 4.7	0.039 ± 0.02	12
Sulfonamide	14.90 ± 1.9*		8
Carbamide	17.40 ± 2.2*	0.095 ± 0.05	12

*Significantly different from DEET at the 95% level.

Table 3

Repellent Evaporation Rates and Dry Protection Times

Repellent	Boiling Points (°C)*	Evaporation Rate at 30° C ($mg/cm^2/hr$)	DPT (hrs)
6-12	94-96° C (0.5)	56.5×10^{-3}	$3.5 \pm .5$
DEET	104-106° C (0.5)	22.6×10^{-3}	6.68 ± 0.81
SRI-6	116-124° C (0.5)	9.31×10^{-3}	8.5 ± 4.7
Sulfonamide	110-120° C (0.5)	8.25×10^{-3}	14.9 ± 1.9
Carbamide	118-122° C (0.5)	2.23×10^{-3}	17.4 ± 2.2

*Parentheses indicate pressure (mm Hg)

Table 4

Molecular Structure of Repellents

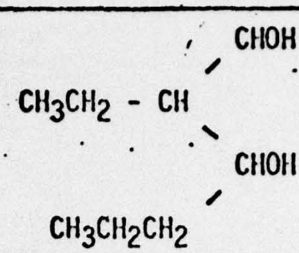
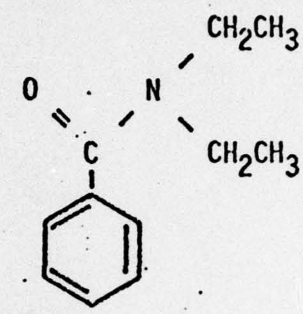
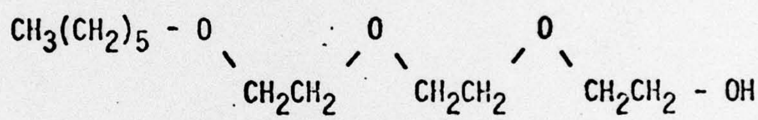
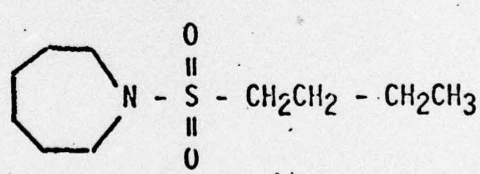
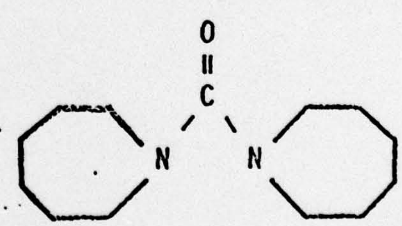
6-12	
DEET	
SRI-6	
Sulfonamide	
Carbamide	

Fig 1

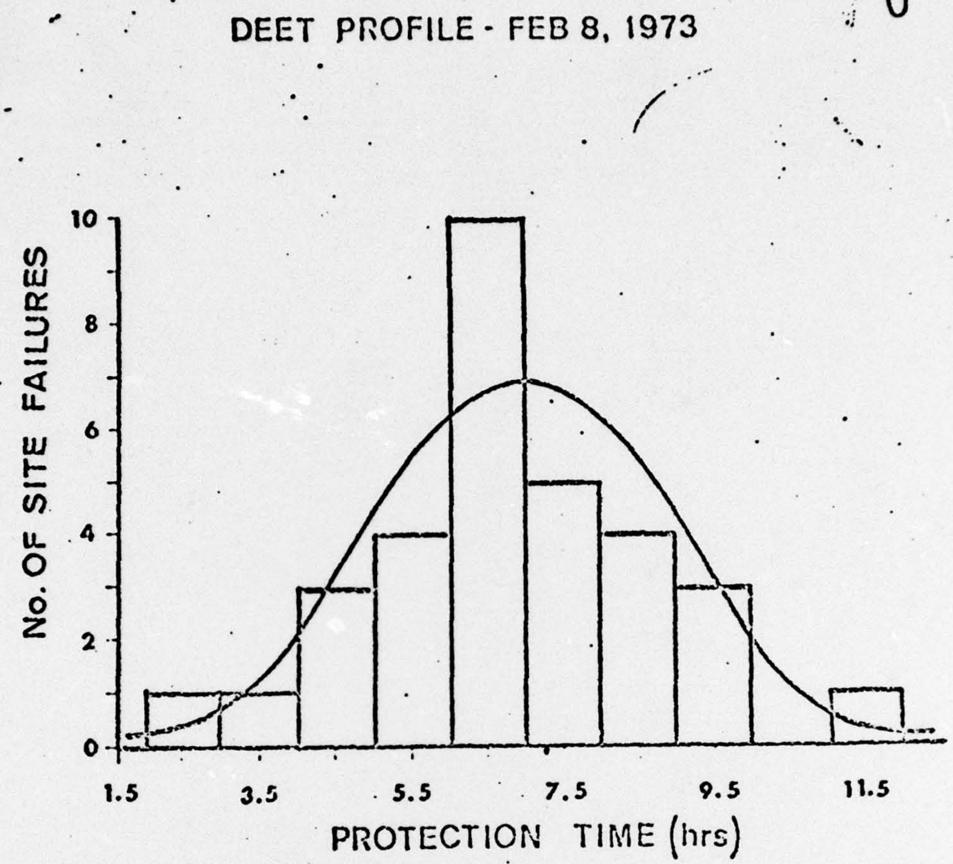


Figure 1

Profile of repellent dry protection times for 32 subjects tested on the same day. DEET was applied at 0 32 mg/cm^2.

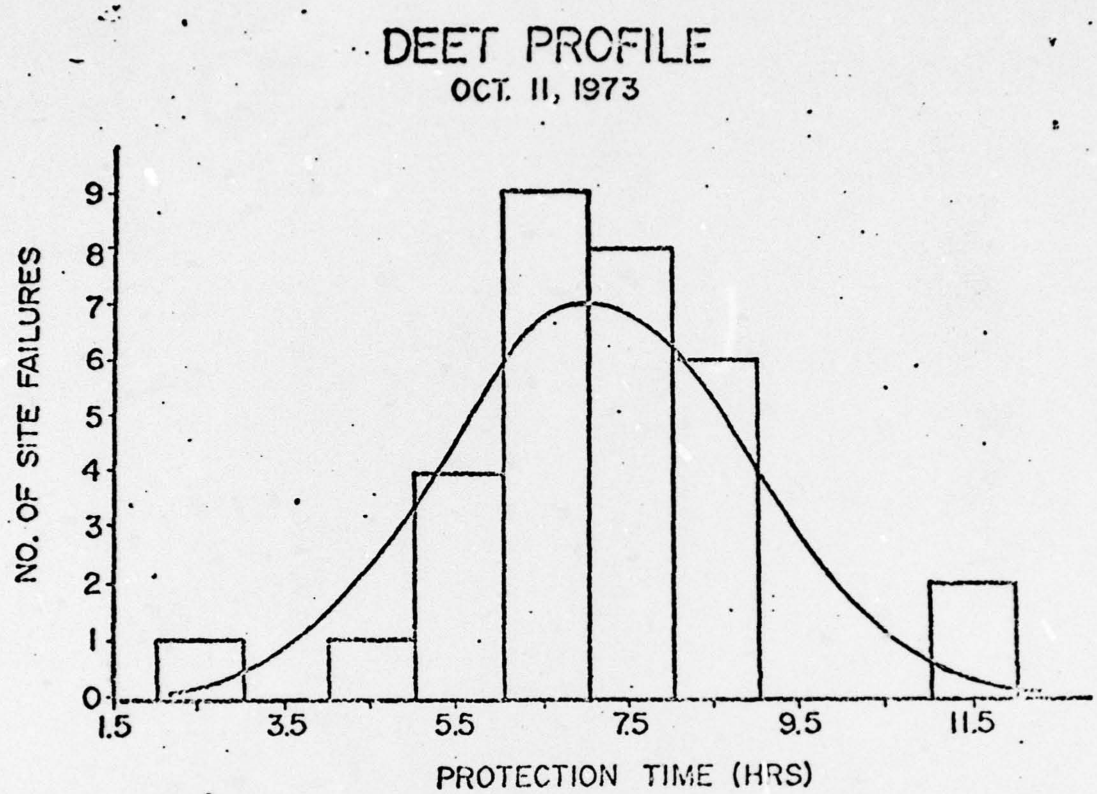


Figure 2

Profile of repellent dry protection times for 32 subjects tested on the same day. DEET was applied at 0.32 mg/cm^2.

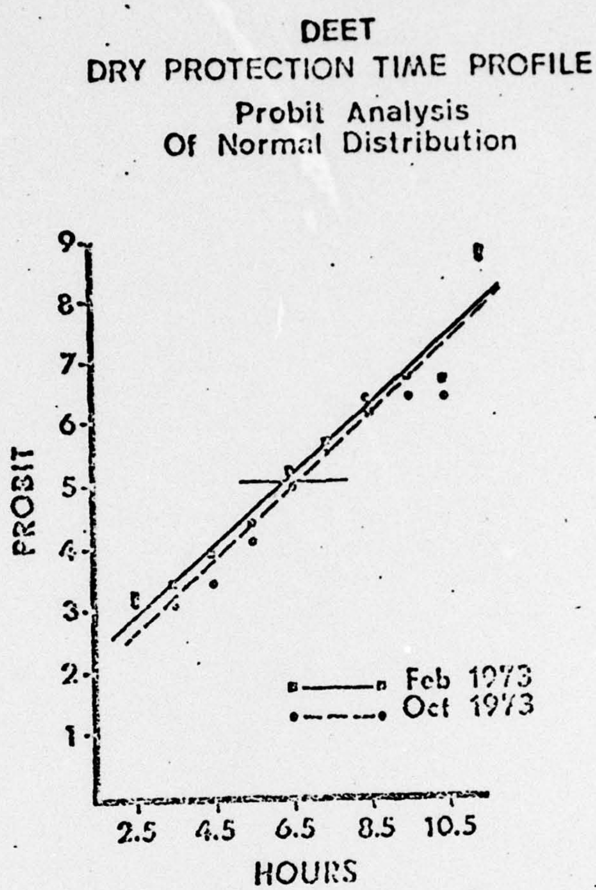


Figure 3

Probit analysis of dry protection time profiles where a normal distribution is indicated by the linearity of the probit points.

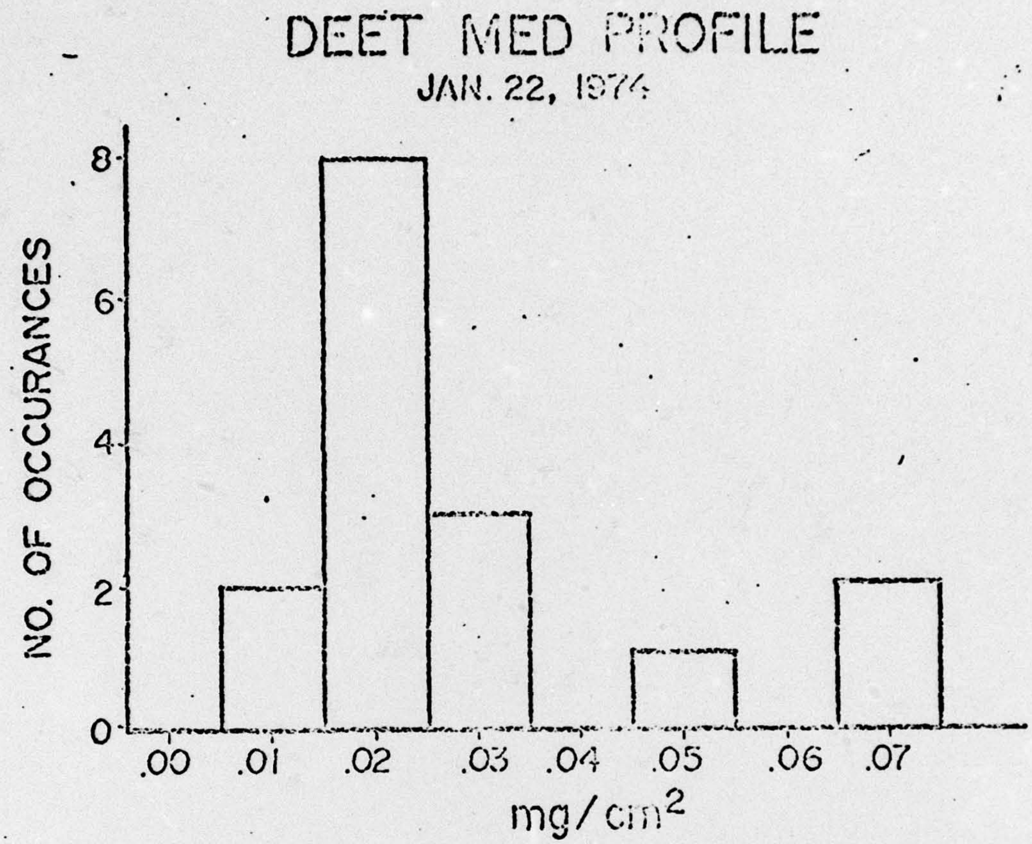


Figure 4

Profile of the minimum effective dose of the repellent DEET for 16 subjects.